



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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For : DNA Demethylase Antisense and Chemotherapy Combination

DECLARATION

I, Nicolas Torno, c/o Cabinet Regimbeau, 20 rue de Chazelles, 75017 Paris (France), hereby declare that I am well acquainted with the French and English languages and hereby certify that to the best of my knowledge and belief the following is a true translation of French priority n° 0202879 filed on March 7, 2002.

All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Date : March 23, 2007


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The present invention relates to a combination product comprising an antisense of the gene encoding MBD2 demethylase and at least one agent used in antitumor chemotherapy, in particular bleomycin, for simultaneous, separate or prolonged use for treating proliferative and inflammatory diseases, in particular for treating cancer.

DNA methylation is an important epigenetic mechanism which regulates gene expression (1-4). One of the characteristics of cancer cells lies in an aberrant methylation scheme (5). Two contradictory changes in the methylation scheme have previously been documented, namely the hypermethylation of selected genes (6) and overall hypomethylation (7).

At the current time, it is not entirely known which mechanisms are responsible for the changes observed in DNA methylation. It is possible that these changes are a consequence of the deregulation of expression of the various components of the DNA methylation machinery (8). The DNA methylation machinery is made up of DNA-methyltransferase (9), of demethylases (10, (11) and (12) and of methylated DNA binding proteins (MBDs) which interpret the DNA methylation signal (13). A certain number of observations support the hypothesis that deregulation of the maintenance DNA-methyltransferase DNMT1 plays an important role in tumorigenesis (14), (15) and (16). An important question is therefore whether other components of the DNA methylation machinery are themselves also essential in nature for cell transformation (8) and (17).

It has been proposed that the hypermethylation of tumor suppressor genes would serve as a mechanism for silencing

essential genes which inhibit various steps of tumorigenesis. The consequence of this hypermethylation will be to promote the process resulting in cell transformation (18). Methylated cytosines are specifically
5 recognized by MBDs (13) and (19-21), which associate with corepressors such as Sin3A, recruit histone deacetylases for methylated genes (22-26) and can be found in known transcription repression complexes, such as Mi2 (27).

10 Mecp2, which is the most well-characterized member of the family, is probably not very important as regards the silencing of genes during transformation, since it is not expressed in cancerous cells (20). Other candidate proteins must be involved. A recently characterized methylated DNA
15 binding protein, MBD2, is an interesting candidate for the reasons disclosed below.

First of all, the MBD2 cDNA has been cloned from a cancer cell line cDNA library (28), and it has been found that it
20 is expressed in breast cancer samples and cell lines (29). Secondly, the protein is involved not only in suppression of the gene by a mechanism similar to that which is presented for Mecp2 (24) and (27), but it has also been found that it also carries a demethylase activity (28).

25 The demethylase activity has previously been purified from a human non-small cell lung carcinoma line A549 (12), and it was similarly found that transfection of the embryonic cell line P19 with the Ha-Ras protooncogene results in an
30 increase in the demethylase activity (30). It is not impossible that an increased demethylase activity is associated with tumorigenesis, and that it could in part be responsible for the overall hypomethylation observed in cancer cells (17). Thus, Mbd2/demethylase could be part of
35 the machinery involved in mediating or interpreting the two contradictory changes associated with the DNA methylation

scheme in cancer cells, namely hypermethylation and hypomethylation.

Although this demethylase activity has been contested by certain groups (24), it has been shown that Mbd2b/demethylase obtained by recombination, expressed in a heterologous cell line SF-9, exhibits demethylase activity. In addition, the cotransfection of Mbd2b/demethylase and of methylated plasmids causes demethylation of these plasmids, and the forced expression of Mbd2b/demethylase in PANC-1 cells results in demethylation and in the induction of the endogenous MUC-2 promoter.

The present invention provides the elements demonstrating that Mbd2/demethylase is effectively expressed in cancer cells, and that it is essential to the growth of tumor cells in culture and in vivo.

No combination of gene therapy and of chemotherapy, consisting in combining an agent used in antitumor chemotherapy with a gene therapy based on an antisense of a gene involved in the level of DNA methylation, such as that of MBD2/demethylase, has been described in the state of the art.

Now, by combining a chemotherapy using bleomycin and the intratumor electrotransfer of a plasmid encoding the genetic antisense of the human DNA demethylase MBD2, a powerful synergistic effect in the treatment of tumors is obtained. The main advantage of the invention is therefore its surprising effectiveness since, if one considers the complete cure rate for tumors, it is 10% using gene therapy by electrotransfer of the MBD2 demethylase gene alone, and also 10% with bleomycin chemotherapy alone, and this rate increases to 40% using the combination of the two treatments: gene therapy and chemotherapy.